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13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

In a significant subset of human breast cancers (HBC), as in other solid tumors, gene amplification and over expression is an important mechanism for oncogene activation. Amplification of the 8p11-p12 region has been found in about 10-15% of human breast cancer and may be associated with poor prognosis. We have used fluorescence *in situ* hybridization (FISH), Southern blot analysis, northern blot analysis, and chromosome 8-specific cDNA array to map this amplicon in 3 breast cancer cell lines, SUM-44, SUM-52 and SUM-225, and identified several novel candidate genes including TC-1 (C8ORF4) and FLJ14299. The specific aims as outlined in this proposal will help us to better understand the biological function and genetic pathway of new target genes of this amplicon and identify better prognostic and predictive markers for HBC. We have made significant progress in the past year in characterizing two novel genes, TC-1 and FLJ14299, within 8p11-12 amplicon. Overexpression of TC-1 in normal human mammary epithelial cells promotes cell growth and anchorage-independent growth. Furthermore, Knockdown TC-1 expression with siRNA suppressed the cell proliferation in breast cancer. Detailed analysis of amplification pattern of 8p11-12 genes in primary breast cancer demonstrated that TC-1 and FLJ14299 are most commonly amplified.

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Introduction:

Gene amplification plays an important role in tumorigenesis of human neoplasia, including breast cancer. Oncogenes, such as ERBB2 , CCND1 and C-MYC have already been identified as amplification targets associated with development, progression, or metastasis of breast cancer(1-4). However there are several amplified regions where target genes have yet to be characterized. One of these is the 8p11-p12 region, which has been shown to be amplified in about 10-15% of human breast cancer (HBC). Using chromosome comparative genomic hybridization (CGH) and array CGH, overlapping amplicons were found to be centered around chromosome 8p11.2 in 3 breast cancer cell lines developed in Dr. Ethier's laboratory, SUM-44, SUM-52 and SUM-225. Thus, these 3 breast cancer cell lines are ideally suited for studies aimed at developing a better understanding of this genomic region in HBC, and for identifying and characterizing novel candidate oncogenes present at these loci. We have used fluorescence *in situ* hybridization (FISH), Southern blot analysis, northern blot analysis, and chromosome 8-specific cDNA array to map this amplicon in the three cell lines, and identified several novel candidate genes including TC-1 (C8ORF4), FLJ14299 and others(5). TC-1 is a novel gene highly expressed in thyroid cancer and some fraction of breast cancers. FLJ14299 contains a C2H2-like motif, which is also present in several tumor-related genes. Aberrant expression of TC-1 and FLJ14299 could be related to development and progression in breast cancer. The specific aims as outlined in this proposal will help us to better understand the biological function and genetic pathway of new target genes of this amplicon and identify better prognostic and predictive markers for an important subset of breast cancer.

Body:

Task 1. To test the mechanistic significance of two novel genes, *FLJ14299* and *TC-1,* found to be amplified and over expressed in the SUM-44, SUM-52 and SUM-225 breast cancer cell lines (Months 1-20)

In order to establish causal links between genes over expressed in breast cancer cells and their function in cell transformation, it is important to either over express those genes in normal human mammary epithelial cells, or block their expression in appropriate breast cancer cells. In this way, one can determine the connection between expression of particular genes, and the expression of a specific transformed phenotype.

We used bicistronic retroviral expression vectors (PNG3000) to express full-length TC-1 and FLJ14299 cDNAs in normal human mammary epithelial cells (MCF10A). MCF10A were infected with virus in the presence of polybrene for 24 hours. Clonal or polyclonal populations of cells resistant to Puromycin were selected and tested for the

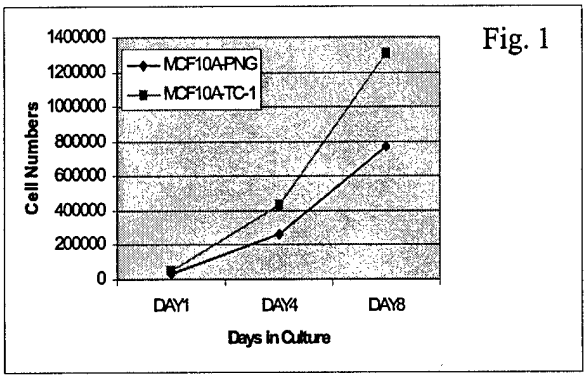
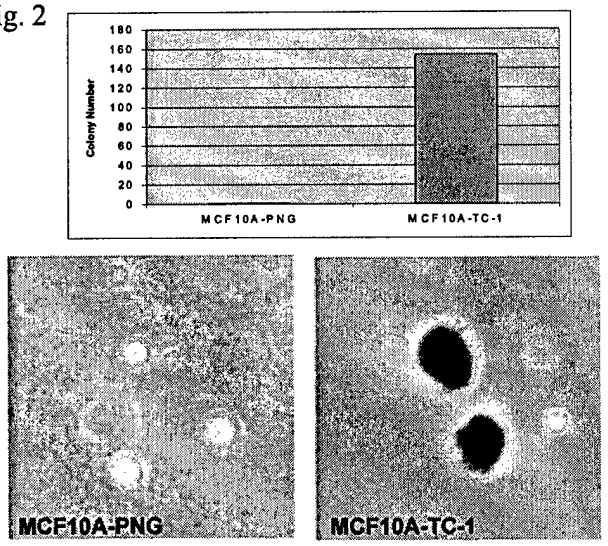


Fig. 1

expression of the inserted genes either by Northern blot and/or RT-PCR. The expression levels of exogenous TC-1 and FLJ14299 in MCF10A-TC-1 and MCF10A-FLJ14299 cells are same or higher as that observed in breast cancer cells in which the gene is amplified and over expressed. Overexpression of TC-1 in MCF10A cells enhanced cell proliferation in tissue culture (Fig.1). More important, TC-1 overexpression significantly promoted colony formation in soft agar assay relative to control vector alone (Fig.2). These results support the notion that TC-1 can facilitate cell growth and anchorage-independent growth associated with transformed phenotypes. Overexpression of FLJ14299 in MCF10A cells did not enhance cell proliferation and anchorage-independent growth.

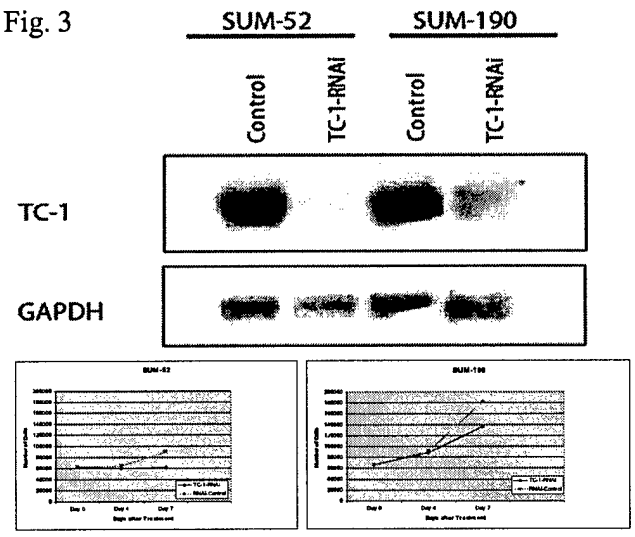
Fig. 2



TC-1 expression enhances anchorage-independent growth in vitro. A immortalized human mammary epithelial (HME) cell line, MCF10A, was infected with a control retrovirus (vector PNG) and a retrovirus encoding TC-1 to generate the cell lines indicated. Ectopic TC-1 overexpression enhances anchorage-independent growth in vitro.

We next assessed the biological functional of TC-1 in HBC cells using RNAi experiment. We chose SUM-52 and SUM-190 cell lines because these cells have the highest TC-1 mRNA expression level among our panel of human HBC cell lines tested. Two 21-nucleotide double-stranded RNA corresponding to human TC-1 was introduced into SUM-52 and-190 cells. A significant reduction of TC-1 mRNA expression level was observed 48 hrs after the TC-1siRNA-175 treatment. The knockdown levels with TC-1 siRNA in SUM-52 were lower than that in SUM-190. The consequences of decreased TC-1 expression on cell growth were evaluated. siRNA targeted TC-1 suppressed the proliferation of SUM-52 and SUM-190 cell lines (Fig.3). This suggests that decreased TC-1 expression in breast cancer cell lines impairs their proliferation.

Fig. 3



Specific inhibition of TC-1 expression by siRNA in SUM-52 and -190 cells. Breast cancer cells were transfected with control siRNA oligos and oligos specific for TC-1 mRNA. Total RNA were prepared 48 h after transfection and TC-1 expression levels were detected by Northern blot and/or quantitative RT-PCR. Bottom graphs are the growth curve of SUM-52 and SUM-190 breast cancer cell lines with TC-1 siRNA treatment.

Task 2. To detect potential downstream target genes of *TC-1* and *FLJ14299* overexpression using human cancer array and to investigate their particular tumor-related biological phenomena (Months 8-36)

TC-1 is a novel gene that was originally cloned from suppression subtractive hybridization between papillary thyroid carcinoma and its surrounding normal thyroid tissue(6). Recently, the structural characterization of the TC-1 protein revealed it is a natively disordered protein(7). Disordered proteins have been suggested to play roles in cell-cycle control, signal transduction, transcriptional and translational regulation. *FLJ14299* encodes a hypothetical protein that contains one C2H2 finger domain. C2H2 zinc finger domain is a nucleic acid-binding protein structure that is widely found in nuclear proteins involved in transcriptional regulation. In order to explore and identify TC-1 and FLJ14299 downstream genes, we used Affymetrix array to profile the up- and down-regulation genes by TC-1 and FLJ14299. Human cancer array have been replaced by Affymetrix array because it contains more than 22,000 probe sets compared with ~3000 probe sets of human cancer array. The top 20 up-and down-regulated genes of TC-1 and FLJ14244 are shown in table 1. Validation and bioinformatic analysis of the Affymetrix array results are ongoing.

Table 1: Top-20 up-regulation genes by TC-1 and FLJ14299

Up-regulated genes of TC-1	fold change	Up-regulated genes of FLJ14299	fold change
hypothetical protein PRO1966	73.33	H2A histone family, member O	11.71
glycine amidinotransferase	16.01	solute carrier family 25 (mitochondrial oxodicarboxylate carrier), member 21	6.69
solute carrier family 25, member 21	7.48	kallikrein 10	5.97
insulin induced gene 1	5.74	solute carrier family 12 (sodium/potassium/chloride transporters), member 1	5.87
solute carrier family 12, member 1	5.41	kallikrein 6 (neurosin, zyme)	5.79
protease inhibitor 3, skin-derived (SKALP)	5.38	B-cell linker	5.42
KIAA0501	5.11	KIAA0810 protein	5.11
kallikrein 10	4.85	insulin induced gene 1	4.75
keratin 6B	4.78	claudin 8	4.73
insulin induced gene 1	4.67	kallikrein 8 (neuropsin/ovasin)	4.67
matrix Gla protein	4.66	cystatin E/M	4.51
hypothetical protein MGC13053	4.64	E74-like factor 3 (ets domain transcription factor, epithelial-specific)	4.49
3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	4.57	H2B histone family, member Q	4.38
cDNA DKFZp566A193	4.46	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	4.31
paired-like homeodomain transcription factor 2	4.33	CED-6 protein	4.26
keratin 16 (focal non-epidermolytic palmoplantar keratoderma)	4.19	hypothetical protein MGC4309	4.2
hypothetical protein DKFZp434N1235	3.94	paired-like homeodomain transcription factor 2	4.05
FLJ20931	3.77	E74-like factor 3 (ets domain transcription factor, epithelial-specific)	3.98
keratin 17	3.75	kallikrein 7 (chymotryptic, stratum corneum)	3.96
isopentenyl-diphosphate delta isomerase	3.73	GABA(A) receptors associated protein like 3	3.92

Task 3. To determine the amplification and overexpression pattern of 8p11-12 genes in primary breast cancer and to determine their associations with tumor phenotype and prognosis (Months 6-36)

To determine the amplification pattern of 8p11-12 genes in primary breast cancer, quantitative PCR analysis was carried out using genomic DNA obtained from microdissected breast cancer specimens derived from our frozen breast cancer bank. The PCR experiments were performed using primers specific for *FLJ14299, LSM1, FGFR1* and *TC-1,* as these genes span the 8p11-12 amplicon detected in the cell lines(5). Of the 32 breast cancers examined, 8 showed evidence of high level amplification (greater than 4-fold) in at least part of the 8p11-12 region. Interestingly, *TC-1* and *FLJ14299* were most commonly amplified, while *FGFR1* was only found to be greater than 4-fold amplified in 1 of 32 primary breast cancers (Table 2). These results suggest that genes such as TC-1 and FLJ14299 flanking the *FGFR1* locus may be of greater significance in

breast cancers. The analysis of expression pattern of these genes and their associations with clinical parameters are ongoing.

Table 2. Greater than 4-fold Copy Number Increase Assessed by Quantitative Genomic PCR Analysis of Microdissected Primary Breast Cancer Specimens

FLJ14299	LSM1	FGFR1	TC-1
5/32	3/32	1/32	6/32

Key Research Accomplishments:

1, Novel gene TC-1 of 8p11-12 amplicon can facilitate cell growth and anchorage-independent growth.

2, Knockdown TC-1 expression with siRNA in breast cancer cells suppressed the proliferation.

3, Quantitative PCR analysis revealed that genes such as TC-1 and FLJ14299 that flanking the FGFR1 locus may be of greater significance of breast cancer.

Reportable Outcomes:

Manuscripts:

Yang Z-Q, Albertson D and Ethier S. Genomic organization of the 8p11-12 amplicon in three breast cancer cell lines. Cancer Genetics and Cytogenetic. In press

Abstracts:

1. Yang Z-Q, Ray M, Albertson D and Ethier S. Characterization of the novel candidate oncogene TC-1 in breast cancer and knockdown using siRNA. 2004 American Association for Cancer Research Annual Meeting in Orlando, Florida. March 27-31, 2004.
2. Yang Z-Q, Ray M, Albertson D, Kleer C and Ethier S. Genomic organization of the 8p11-12 amplicon in human breast cancer. SKCC Genomics, Signaling and Tumor Targeting Conference in San Diego CA, February 16-18, 2004(Oral and poster presentation)

Conclusions:

We have made significant progress in the past year in characterizing two novel genes, TC-1 and FLJ14299, within 8p11-12 amplicon of breast cancer. Overexpression of TC-1 in normal human mammary epithelial cells promotes cell growth and anchorage-independent growth. Furthermore, Knockdown TC-1 expression with siRNA suppressed the cell proliferation in breast cancer. Detailed analysis of amplification pattern of 8p11-12 genes in primary breast cancer demonstrated that TC-1 and FLJ14299 are most commonly amplified.

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APPENDICES:

Cancer Genetics and Cytogenetics in press paper: Yang Z-Q, Albertson D and Ethier S. Genomic organization of the 8p11-12 amplicon in three breast cancer cell lines.

Genomic organization of the 8p11-12 amplicon in three breast cancer cell lines

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Abstract

Amplification of chromosomal regions leads to an increase of DNA copy number and expression of oncogenes in human breast cancer (HBC). Amplification of the 8p11-12 region occurs in 10-15% of primary, uncultured HBCs. In our panel of 11 breast cancer cells, three cell lines, SUM-44, SUM-52 and SUM-225 have overlapping amplicons in 8p11-12 region. In order to characterize genome structure of the amplified regions, we performed fluorescence *in situ* hybridization (FISH) using 8p11-12 BAC clones in the three cell lines. The results revealed that the 8p11-12 amplicon has a highly complex structure and that *FGFR1* is not in the common core-amplified domain in 3 breast cancer cell lines with the amplicon. These three cell lines provide good models for genetic and functional studies of candidate oncogenes of 8p11-12 region

1. Introduction

Genomic amplification is often observed in many types of human tumors, including human breast cancer (HBC). Oncogenes such as *ERBB2* (17q12), *CCND1* (11q13), and *C-MYC* (8q24), are activated by amplification and play a role in the development of some fraction of HBCs [1-4]. Recently detailed analyses of genomic structures and sequences of amplified regions such as those found at 11q13, 17q12-q23, and 20q12 have revealed that amplicons have complex patterns and frequently involve non-syntenic as well as syntenic DNA

from the same chromosomal region and can harbor multiple genes likely to be associated with tumorigensis [5-7]. In breast cancer, the 11q13 amplicon can vary in size from less than 1Mb to 4.5 Mb. This amplicon includes a number of candidate oncogenes including *CCND1* and *EMS1*, which can be amplified independently of each other [2, 5]. Similarly there appears to be two distinct regions on chromosome 17q that become amplified in breast cancer; the 17q11 region that harbors *HER-2* and other candidate oncogenes, and the 17q23 region that has been recently described by different laboratories [6, 8].

Tumor cell lines are good models for fine mapping of amplified genomic regions, and for functional studies of candidate oncogenes because their molecular and cytogenetic aberrations and biological properties reflect distinct subsets of primary tumors. Over the past several years, we have developed a novel panel of HBC cell lines that are ideally suited for elucidating molecular biologic characteristics of breast cancer [9]. In our panel of 11 breast cancer cell lines, we found 3 cell lines, SUM-44, SUM-52 and SUM-225 that have overlapping amplicons centered around chromosome 8p11.2. Amplification of the 8p11-12 region occurs in 10-15% of primary, uncultured HBCs [10], and fibroblast growth factor receptor 1 (*FGFR1*) has long been considered to be the best candidate oncogene at that locus. However, the exact involvement of this receptor in the progression of the cancer is unclear because it is not consistently present in the core-amplified domain and is not always overexpressed when amplified. Identification and characterization of amplified regions can provide

important insights into the pathogenesis of breast cancer, and can lead to the identification of targets for novel therapeutics. In this report, we describe in detail the genomic structure of the 8p11-12 amplicon with molecular cytogenetic analysis. Like other amplicons identified in breast cancers, the 8p11-12 amplicon has a highly complex structure, and *FGFR1* is not in the common core-amplified domain in 3 breast cancer cell lines with the amplicon.

2. Results and discussion

Our previous chromosome and array comparative genomic hybridization (CGH) studies demonstrated that three of our breast cancer cell lines, SUM-44, SUM-52 and SUM-225 exhibited high-level amplifications at 8p11-12 [9, 11] Furthermore, the SUM-225 cell line contains a separate amplicon at 8q11. The array CGH results and the detailed map of the 8p11-12 and 8q11 amplicon in SUM-44, 52 and 225 based on the April 2003 freeze of the human genome sequence (UCSC) is shown in Table 1.

In an effort to more carefully define the genomic structure of the amplicons in these three cell lines, and to better understand the mechanistic basis for the copy number increases observed in them, FISH analyses were carried out using BAC probes that map to the 8p11-12 and 8q11 regions. We selected five commercially available BACs from the completed chromosome 8 sequence as probes for the 8p11-12 region: RP11-701H6 (*ADRB3* locus), RP11-350N15

(*FGFR1* locus), RP11-723D22 (*TACC1* locus), RP11-44K6 (*INDO* locus) and RP11-470M17 (*TC-1* locus) (Fig.1). One BAC, RP11-217N16, from the 8q11.1 region was also used in the SUM-225 line. A chromosome 8 centromeric probe, CEP8, was used as a control. Metaphase chromosome slides were prepared from the SUM series of HBC cell lines using standard methods. Chromosomal *in situ* suppression hybridization and fluorescent detection of hybridization signals were carried out as described previously [12]. The copy number and molecular organization of the region of interest were assessed according to the hybridization patterns observed on both metaphase and interphase chromosomes.

In the SUM-44 cell line, all 8p11-12 BACs yielded clustered FISH signals on two marker chromosomes (Fig. 2). In addition, two apparently normal copies of chromosome 8 were present in most metaphase spreads. By contrast, only four FISH signals on most metaphase and interphase spreads were detected with the centromeric (CEP8) probe. In metaphase spreads, the hybridization pattern of the BAC probes and CEP 8 signals were always located on the same marker chromosomes with 8p11-12 amplification. FISH combined with array CGH analysis revealed the amplified 8p11-12 region in SUM-44 to be intrachromosomal and to involve several megabases of syntenic sequences (Fig. 1 and Fig. 2). Based on the FISH hybridization pattern and our previously published karyotype of SUM-44 [13], we suggest a simple primary structural model of the 8p11-p12 amplicon in this cell line. During the tumorigenic process,

the 8 p11-12 region of one chromosome 8 underwent *in situ* amplification resulting in a large tandem duplication of the region. Subsequently the chromosome containing the 8p11-12 amplified chromosome and the normal chromosome 8 were duplicated resulting in two normal chromosome 8s and two chromosome 8s with the amplicon.

FISH patterns from SUM-225 cells resembled those from SUM-44 cells in that copy number increases appear to have resulted from the generation of clustered DNA amplification. However, the regions of focal gene amplification in SUM-225 differ from those of SUM-44 cells. In the SUM-225 cell line, FISH hybridization (Fig. 3) and array -CGH (Table 1) analysis revealed two separate amplified regions; one at 8p11-p12 and a second at 8q11. In metaphase spreads, clustered FISH signals obtained with the 44K6 and 470M17 probes were present on 2-4 marker chromosomes (Fig. 3, top left panel) and more than 18 FISH signals were counted in interphase spreads, while BAC probe 723D22 (*TACC1* locus) yielded 7 to 12 signals in metaphase and interphase chromosome spreads. In contrast, probes 701H6 (Fig. 3, bottom right panel) and 350N15 yielded essentially background signals. Interestingly, BAC probe 217N16, located at 8q11.1, also showed high-level amplification in metaphase and interphase nuclei (Fig. 3, bottom left panel), and clustered FISH signals were also observed in 3-6 marker chromosomes in each metaphase spread. Two-color FISH with 470M17 and 217N16 revealed the 8q11 and 8p11probes have coamplified FISH signals in 2 to 4 marker chromosomes while the 8q11 probe

has independent amplified signals in 1-3 other marker chromosomes. The BAC probe 217N16 from 8q11.1 actually overlaps with the centromere region, and therefore, it is not surprising that the centromeric CEP8 probe also showed increased signals on several marker chromosomes (Fig. 3, top right panel). This suggests that SUM-225 cells share a small common region of gene amplification with the SUM-44 and SUM-52 cells in the 8p11 region, and that they also have a separate unique region of gene amplification across the centromere involving the proximal 8q11 region. Because the 8q11 amplicon has the highest level of amplification in SUM-225 cell line, it may also harbor uncharacterized breast cancer genes. As we can only look at the end product of the amplification process, we do not know if the two amplicons within 8p11 and 8q11 regions of SUM-225 were initiated as a large amplified DNA fragment followed by a secondary rearrangement, resulting in two separate amplicons, or if the two domains were co-amplified in some marker chromosomes and independently in others.

The molecular cytogeneic alterations present in the SUM-52 cells are considerably more complex than for the SUM-44 or SUM-225 cell lines. Previous chromosome banding analysis showed SUM-52 had complex karyotypes with multiple numerical and structural aberrations [14]. The representative karyotype has one normal chromosome 8 with many marker chromosomes (http://www.cancer.med.umich.edu/breast_cell/Production/sumlines/karyotypes/Sum-52PEKaryotypes.html). In the FISH hybridization, the signal intensity and

hybridization pattern of the five 8p11-p12 BAC probes in SUM-52 interphase and metaphase spreads were complex and heterogeneous. Twelve to 20 copies of the five BAC probes were detected in metaphase chromosomes and interphase nuclei while eight to 12 copies of the CEP8 probe were observed. In most metaphase spreads, intense signals of BAC probes were present on 1 to 3 marker chromosomes, suggesting clustered duplication of the corresponding genomic segment (Fig. 4, top and bottom right panels). Interestingly, CEP8 signals were not detected on these marker chromosomes. However BAC and CEP8 signals were detected in 2-3 cytogenetically normal chromosomes 8s (Fig. 4, top left panel). In addition, CEP8 signals were detected in 4-8 chromosomes that did not yield signals with 8p11-12 BAC probes (Fig. 4, bottom left panel). These CEP8 signals were generally fainter and smaller than the corresponding normal centromeric signals. The fact that several derivative chromosomes showed only 8p11-12 BAC or CEP8 signals in FISH hybridization, suggests that breaks and translocations in 8p11-p12 likely occurred during the amplification process. Indeed, Adelaide et al, has recently demonstrated that SUM-52 cells have 8p12 amplification and 8p12-pter loss with breakpoints in the NRG1 gene [15]. Thus, unlike SUM-44 and SUM-225 cells, SUM-52 cells have gene amplification as a result of more complex translocation and rearrangement. The presence of nonsyntenic amplified regions of the genome in the SUM-52 and SUM-225 cells suggests that the amplification process resulted from a combination of molecular events.

Gene amplification is a frequent event in human cancers, but little is known regarding the mechanism of gene amplification or how the overall genomic structure that constitutes the amplified DNA is assembled. From the detailed studies of in vitro model systems of drug-resistant cell lines, it is generally agreed that at least two different mechanisms can drive amplification [16-18]. One is a breakage-fusion-bridge (BFB) cycle mechanism that accumulates copies organized as large repeats on a chromosome arm where one normal gene copy maps in non-amplified cells [16, 19]. Second, the amplified DNA can megabase-long extra-chromosomal DNA sequences called double minute. The BFB mechanism has proved relevant to the specific breakage of genomic DNA at fragile sites that are points at which chromosomes break non-randomly under certain specific conditions [18-21]. Coquelle et al. have found that fragile sites trigger intrachromosomal gene amplification and form the boundaries of amplicons [16]. In addition, gene amplification mediated by BFB cycles at fragile sites has been demonstrated in human cancer, such as for the MET oncogene in gastric cancer through FRA7G, and for the RIN gene in oral cancer through FRA11B [22, 23].

The 8p11-q11 region has been reported as a common fragile site [24]. This suggests 8p11-12 and 8q11 amplification in our cell lines appears to have resulted from BFB cycles at the 8p11-q11 fragile site. Our data from FISH, array-CGH and Southern blot analysis indicates that extensive DNA

rearrangement and loss of intervening DNA may have taken place during the evolution of the 8p11-12 amplification in SUM-52 and SUM-225cell lines [11]. Our observations are in line with those made by Adelaide et al., who found the 8p12-p21 region is particularly complex with at least seven different breakpoint targets within the NRG1 gene in breast and pancreatic cancer cell lines [15]. The genesis of such complex abnormalities cannot be fully explained by BFB cycles and likely involves additional breakage and recombination events at both fragile sites and non-fragile site regions.

The results of the FISH studies were consistent with and extended the information gained from the conventional CGH and array CGH analyses performed previously, and confirmed that *FGFR1* is beyond the core-amplified domain in SUM-225 cell line. Detailed expression profiling of the amplicon in our 3 breast cancer cell lines using a chromosome 8 cDNA microarray and northern blot analysis revealed FGFR1 is overexpressed at the message level only in the SUM-44 line [11]. Several genes including *TACC1*, *C8ORF4* (*TC-1*) and other genes within common core-amplified domain have been found to be over expressed in breast cancer cell lines and primary tumors [11, 25]. Our results suggest that the 8p11-12 region, which has a similar complex amplification pattern as those observed at chromosome 20q12, 17q22-q24, and 11q13, may contain more than one important gene. Co-selection, and a synergistic role of two or more genes may occur in the development and progression of some breast cancers. Further studies of the amplification and expression of candidate

breast cancer oncogenes in a large set of primary breast cancers, as well as determination of their function in cell transformation, will be necessary to address the relationship between these genes and breast cancer progression.

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Figure Legends:

Table 1: Mapping profile of array-CGH within 8p11-12 and 8q11 region in 3 cell lines. The BAC clones in array-CGH are ordered by cytoband and base-pair position, and the log2 Ration of each BAC probe are given for each cell lines. Shaded regions indicate ratios greater than or equal to 1.00 as genomic amplification. The BACs position and genes within each BAC clone were

obtained from UCSC database (http://genome.ucsc.edu/, April 2003). ND, not determined

Fig.1. Ideogram of 8p11-12 amplicon in 3 HBC cell lines, SUM-44, SUM-52 and SUM-225. The position of representative genes based on UCSC database (http://genome.ucsc.edu/, April 2003), and BACs used for FISH are presented to the right of the chromosome 8 ideogram. The amplicon in each cell line is based on the results of array-CGH, FISH and southern blot analysis.

Fig. 2. Representative images of FISH analysis of the 8p11 amplicon demonstrates high-level amplification of the 8p11-12 region in SUM-44 HBC cells. The BACs of 8p11-12 region were amplified on two marker chromosomes and in more than 20 signals in interphase cells. There are only four chromosome 8 centromere signals (red) in the displayed metaphases and interphase spreads.

Fig 3. Representative images of FISH analysis of the 8p11 amplicon demonstrates the two separate peaks of amplification in SUM 225 cells. The 44K6 BAC shows 8p11 amplification, however the 701H6 BAC does not show amplification. The centromeric probe and the 217N16 BAC from 8q11 also show amplification of sequences from the opposite side of the centromere at proximal 8q11.

Fig. 4 Representative images of FISH analysis of the 8p11 amplicon demonstrates representative marker chromosomes from SUM-52 metaphase FISH experiments using the 350N15 BAC probe and the centromeric probe (CEP 8). Displayed are: an apparently normal chromosome 8 (top left), a marker chromosome with only the CEP 8 signal (bottom left), and marker chromosomes with multiple signals from the 350N15 BAC probe (top and bottom right).

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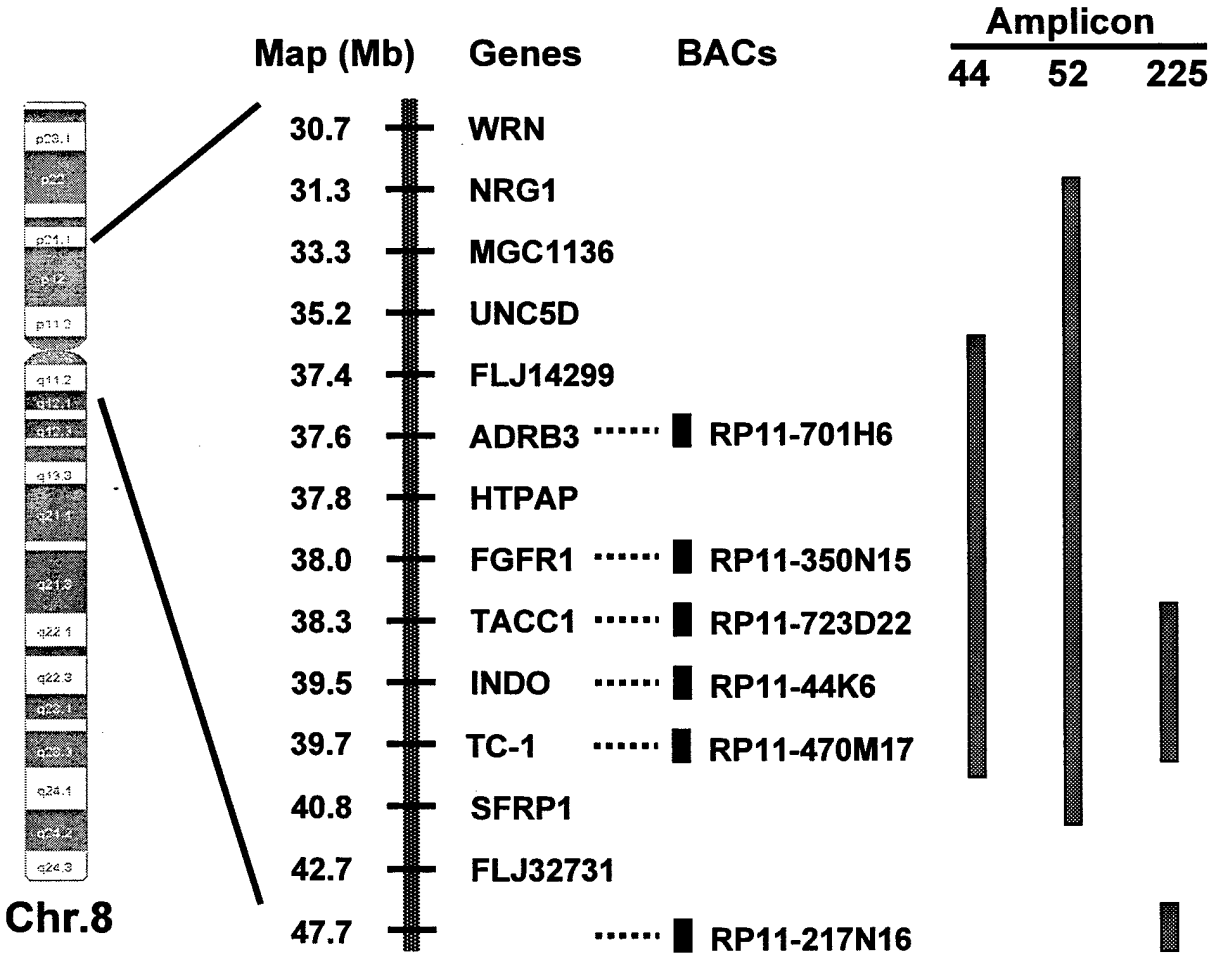
Fig. 1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BACs
Amplicon
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52
225
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WRN
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NRG1
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MGC1136
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UNC5D
37.4
FLJ14299
37.6
ADRB3
RP11-701H6
37.8
HTPAP
38.0
FGFR1
RP11-350N15
38.3
TACC1
RP11-723D22
39.5
INDO
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39.7
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Fig.2

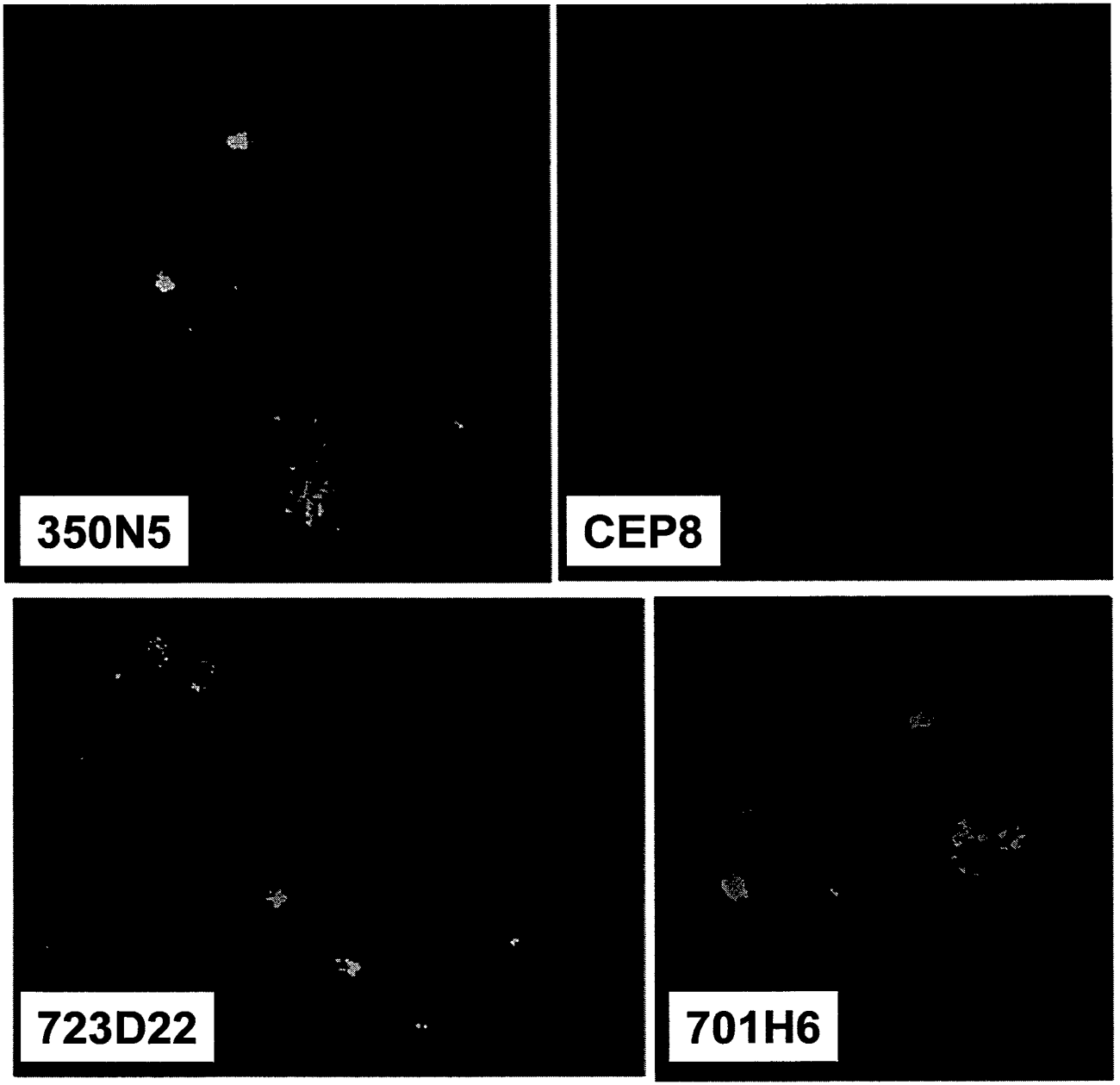

Fig.3

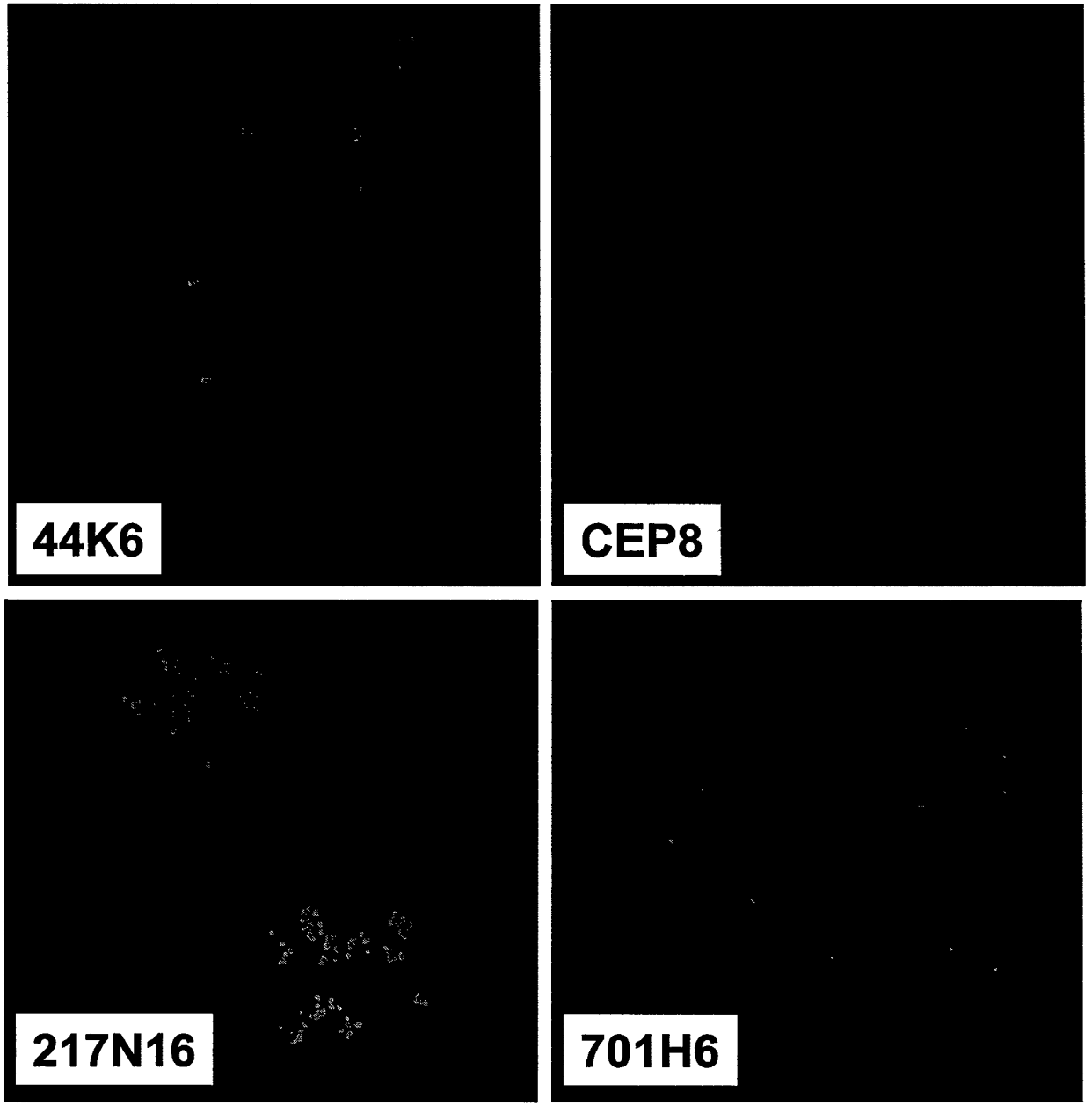

Fig.4

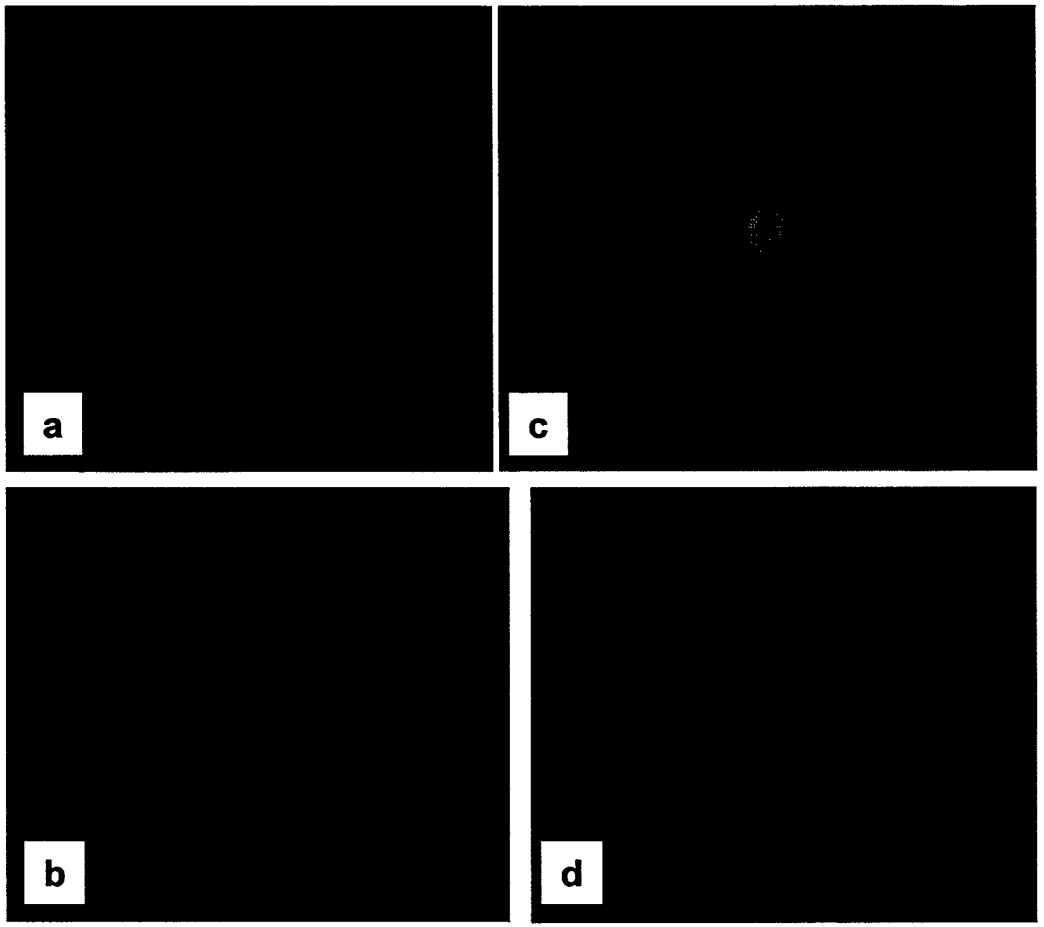

Table 1:

BAC Clone	Cytoband	Gene	Base Start	Base End	SUM-44	SUM-52	SUM-225
RP11-139G9	8p12	WRN	30886709	30928744	-0.72	-0.84	-1.30
CTD-2020E14	8p12		31062838	31063263	-0.73	-0.82	-1.42
RP11-57I3	8p12	NRG1	32268599	32429032	-0.77	1.81	-1.45
RP11-122D17	8p12	NRG1	32435232	32609814	-0.83	1.88	-1.51
RP11-258M15	8p12		33377743	33538816	-0.73	2.34	-1.26
RP11-274F14	8p12	UNC5D	35328931	35329267	-0.85	1.65	-0.49
RP11-237M13	8p12	UNC5D	35433788	35608399	-0.77	1.11	-0.61
RP11-210F15	8p12		36250826	36251210	ND	1.02	-0.64
RP11-265K5	8p12	HTPAP, WHSC1L1, FLJ25409, FGFR	37849071	38026421	ND	1.28	0.69
RP11-100B16	8p12	FLJ25409, FGFR1	37980628	38144207	ND	1.34	0.74
RP11-262I23	8p11.21	INDO	39468496	39667003	1.52	1.43	2.22
RP11-133O7	8p11.21		39578684	39626434	1.89	1.58	2.32
RP11-284J3	8p11.21		40384739	40385032	1.06	1.61	-1.42
RP11-282J24	8p11.21		41386548	41386797	1.52	1.40	-1.32
RP11-64C22	8p11.21		42090855	42125967	0.08	1.47	-1.15
RP11-73M19	8p11.21	FLJ32731, FLJ22242	42724620	42892080	0.07	0.72	-1.22
RP11-12L15	8q11.1		47495880	47647566	-0.03	-0.12	3.36
RP11-217N16	8q11.21		47715320	47715660	0.08	-0.08	3.34
RP11-268N2	8q11.21		48790973	48808994	-0.57	-0.03	-1.07
RP11-149G12	8q11.21		51871742	51872129	-0.39	0.12	-0.78